



10091912.0/3002 #6

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Date: July 26, 2002

By:

Carol A. See  
Carol A. See

PATENT  
Docket No. GC724

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of )

Bott et al. )

Group Art Unit: 1645

Serial No.: 10/091,912 )

Examiner: Unassigned

Filed: March 5, 2002 )

For: High Throughput Mutagenesis  
Screening Method )

Preliminary Amendment

U.S. Patent and Trademark Office  
Box Sequence  
P.O. Box 2327  
Arlington, VA 22202

Sir:

Prior to examination, applicants respectfully request entry of the following amendments.

In the specification:

In the specification after page 19 insert pages 1-2 enclosed herewith.

On page 3, please replace the paragraph starting on line 15 with the following:

Fig. 18 illustrates the amino acid (SEQ ID NO:2) and DNA sequence (SEQ ID NO:1) for cutinase.

On page 10, please replace the paragraph starting on line 14 with the following:

Site-saturation variant libraries were created at amino acid positions corresponding to residue positions 57-66, 68, 85, 86, 88, 125-127, 130, 148-152, 154, 155, 176-183, and 204-211 of SEQ ID NO:2 in *P. mendocina* cutinase, expressed in *Bacillus subtilis* strain 3934. The sequence of the parent cutinase is attached hereto as Fig. 17 (SEQ ID NO:1). Libraries were

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created with the Stratagene Quik-Change™ kit using oligonucleotide primers randomized with NN(G/C) at the target position. Each selected amino acid was randomly replaced with all 19 possible alternatives. Colonies obtained from the variant libraries were selected and placed into 96-well growth plates that contained 100  $\mu$ L/well of MOPS 1A starter medium. The plates were incubated at 37°C overnight with continuous humidified shaking at 260 rpm. A sample from each well was replicated into a Millipore 96-well filter plate which contained 200  $\mu$ L/well of MOPS-Urea medium. 50  $\mu$ L of 45% glycerol was added to each well of the initial growth plates, which then were stored at -70°C.

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REMARKS

Entry of the above amendment prior to examination is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned "Version with Markings to Show Changes Made."

I. Amendments

The specification has been amended in accordance with 37 C.F.R. §1.821 through 1.825 to add the Sequence Listing.

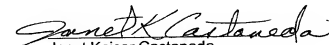
The specification and claims have been amended in accordance with 37 C.F.R. §1.821(d) to add sequence identifiers preceded by SEQ ID NO:.

No new matter is introduced by way of these amendments.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7500.

Respectfully submitted,

Date: 7/24/02

  
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